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Therapeutic Vulnerabilities of Transcription Factors in AML

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ABSTRACT

Acute myeloid leukemia (AML) is characterized by impaired myeloid lineage differentiation, uncontrolled proliferation, and inhibition of proapoptotic pathways. In spite of a relatively homogeneous clinical disease presentation, risk of long-term survival in AML varies from 20% to 80% depending on molecular disease characteristics. In recognition of the molecular heterogeneity of AML, the European Leukemia Net (ELN) and WHO classification systems now incorporate cytogenetics and increasing numbers of gene mutations into AML prognostication. Several of the genomic AML subsets are characterized by unique transcription factor alterations that are highlighted in this review. There are many mechanisms of transcriptional deregulation in leukemia. We broadly classify transcription factors based on mechanisms of transcriptional deregulation including direct involvement of transcription factors in recurrent translocations, loss-of-function mutations, and intracellular relocalization. Transcription factors, due to their pleiotropic effects, have been attractive but elusive targets. Indirect targeting approaches include inhibition of upstream kinases such as TAK1 for suppression of NFκB signaling and downstream effectors such as FGF signaling in HOXA-upregulated leukemia. Other strategies include targeting scaffolding proteins like BrD4 in the case of MYC or coactivators such as menin to suppress HOX expression; disrupting critical protein interactions in the case of β-catenin:TCF/LEF, and preventing transcription factor binding to DNA as in the case of PU.1 or FOXM1. We comprehensively describe the mechanism of deregulation of transcription factors in genomic subsets of AML, consequent pathway addictions, and potential therapeutic strategies.

Introduction

In spite of remarkable progress in the genomic classification and risk stratification of acute myeloid leukemia (AML) by the WHO (1) and ELN (2), 5-year survival rates still stagnate at 30% (SEER data 2009–2015). This is primarily due to the rapid emergence of resistance to standard cytotoxic chemotherapy. The development of effective next-generation therapeutic options against AML depends on mechanistic understanding of AML biology. In the current review, we try to link the genomically and clinically defined ELN leukemia categories with critical transcription factor dependencies. We will highlight several mechanisms of transcription factor deregulation in AML.

Loss-of-function mutations in transcription factors critical for hematopoietic stem and progenitor cell differentiation include RUNX1, and CEBP-α and germline mutations in these transcription factors have also been observed in familial myeloid neoplasms (**Fig. 1**; ref. 3). In addition, loss of function of tumor suppressor p53 results in high-risk AML frequently associated with complex karyotype and chemotherapy resistance.

Disruption of chromatin-associated protein complexes in the case of *MLL*- and *NUP28*-rearranged leukemia can result in altered histone methylation and consequent overexpression of the HOXA cluster of oncogenic transcription factors.

Intracellular cytoplasmic delocalization can inactivate important transcription factors for granulocytic differentiation such as PU.1 in the subset of AML characterized by *NPM1* mutations. Concurrent inactivation of the pro-oncogenic transcription factor FOXM1, another important binding partner of nucleophosmin, may play a role in the clinically observed favorable responses to chemotherapy in this subset of AML patients (**Fig. 1**).

Although molecular aberrations in AML occur in diverse genes and combinations, many of these converge on a finite number transcriptional networks (4). Discovery of the dependencies of the dysregulated transcriptional programs in AML can allow the identification of important therapeutic targets. We will discuss key vulnerabilities in oncogenic and tumor suppressor transcription factors that are dysregulated in AML, including findings from our laboratory.

Transcription Factor Activity Altered by Recurrent Translocations in AML

Transcription factors can be directly involved in translocations or indirectly influenced by dysregulation of coactivators.

HOXA

The *HOX* genes encode a family of homeodomain-containing transcription factors exhibiting temporally restricted expression (5) during hematopoietic cell differentiation, and their deregulation is an important step toward malignant transformation. The mixed lineage leukemia (*MLL*) gene encodes a transcriptional activator that regulates HOX expression through interaction with chromatin-associated protein complexes including menin and the histone H3 lysine 79 (H3K79) methyltransferase DOT1L (6). AML patients with chromosomal translocations or partial tandem duplications involving the *MLL* gene have a poor prognosis that has been linked to enhanced and sustained transcription of *HoxA9* and *HoxA10* (7) by MLL fusion oncoproteins. There is some controversy around the level of addiction to HOXA in *MLL*-rearranged leukemias. *HoxA9*$^{-/-}$–deficient progenitors transduced with MLL-Gas7 (8) are capable of initiating leukemia, and *MLL*-AF9$^{/+}$ knock-in mice with *HoxA9* deletion (9) show no differences in disease development compared with mice with germline *HoxA9*. Other

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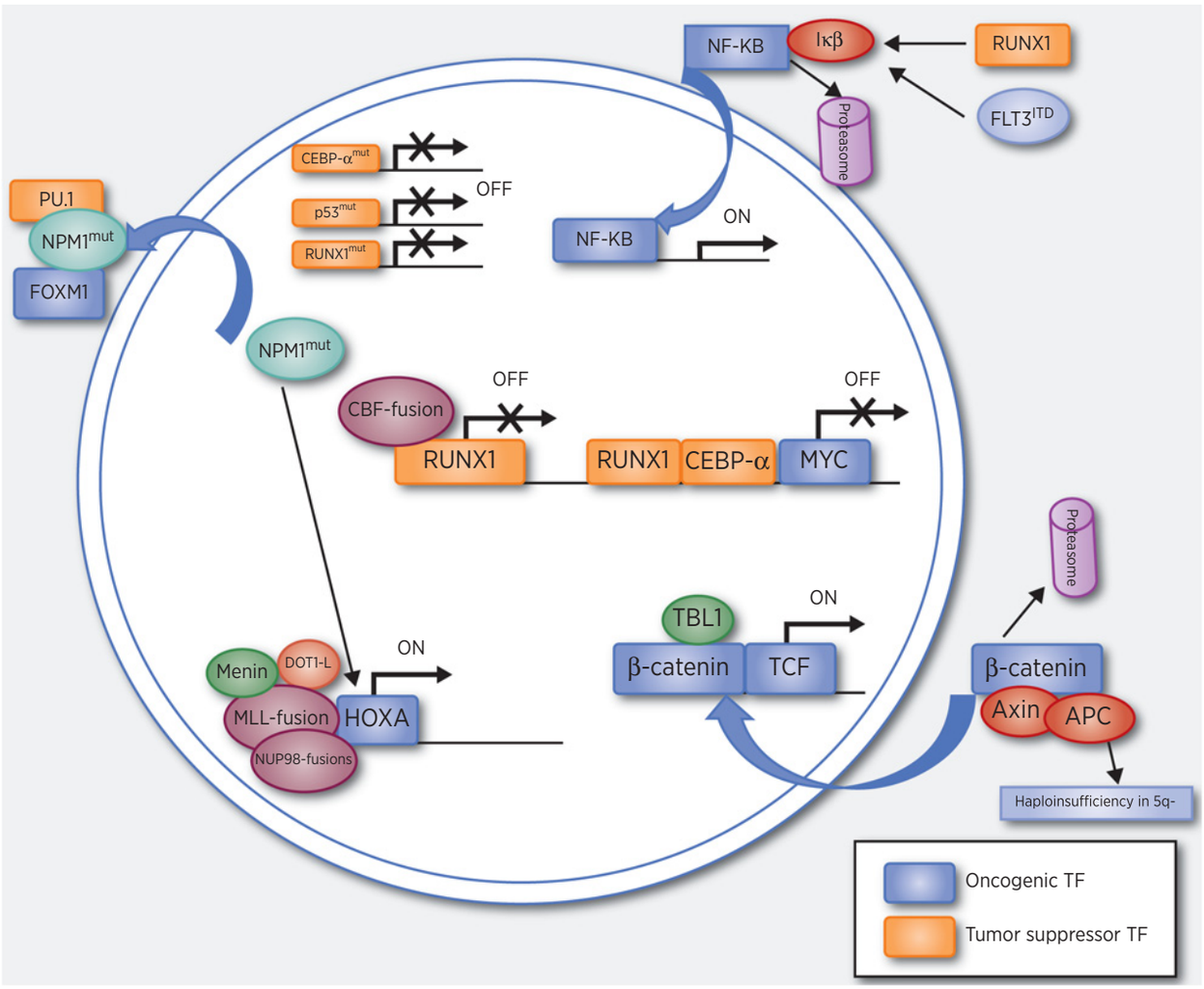

Figure 1.

Mechanisms of transcriptional deregulation in *AML*. Chromosome translocations resulting in MLL and NUP98 fusion oncoproteins result in increased expression of the oncogenic transcription factor *HOXA*. Core bind factor translocations including inv(16) and t(8;21) result in fusion proteins that have a dominant-negative effect on the tumor suppressor RUNX1. *MYC* is transcriptionally silenced by wild-type RUNX and CEBP-α, and mutations/loss of function of these genes leads to its overexpression. Nuclear translocation of NF-kappa by loss of its negative regulator Iκβ is induced by the *FLT3ITD* mutation and loss of function of wild-type RUNX1. Haploinsufficiency of the *APC* and *CK1A1* genes in 5q-AML leads to nuclear translocation of beta-catenin. Once nuclear, it binds to TCF; this complex is stabilized by TBL1, and is transcriptionally active *NPM1* mutations result in cytoplasmic export of PU.1, a critical myeloid differentiation factor. Loss-of-function mutations in *CEBP-α*, *p53*, and *RUNX* results in loss of their tumor suppressor function.

studies show an abrogation of leukemia development with *HOXA9* knockdown in *MLL*-AF9 leukemia (10). It is also known that overexpressing HOXA9 alone does not induce AML in murine bone marrow transplant studies, but requires collaboration with Meis1 (11) although HOXA10 alone can cause AML (12).

Another important but rare subset of AML that is highly dependent on HOXA transcriptional activity is *NUP98*-rearranged AML (13). Although *NUP98* can be directly fused to *HoxA9*, it has been shown that irrespective of the binding partner, NUP98 fusion proteins directly interact with nonspecific lethal (NSL)/MLL1 histone-modifying complexes and colocalize on chromatin where they are associated with HOX gene promoter regions. *NUP98*-rearranged leukemias show elevated expression of HOXA and HOXB cluster genes, and mouse model systems have recapitulated this high-level expression.

In addition, several publications support high expression of multiple members of the HOXA and HOXB clusters in *NPM1*-mutant AML (14, 15). Using allele-specific gene editing and treatment with nuclear export inhibitors, Brunetti and colleagues (16) demonstrated a dependency of HOX expression, and AML cell differentiation and proliferation on the aberrant cytoplasmic localization of nucleophosmin (NPM).

The DOT1L inhibitor EPZ-5676 inhibits methylation of H3K79 resulting in downregulation of *HOXA9*, which correlated with the anticancer activity of this drug in MLL-rearranged AML xenografts (17). The much-anticipated phase I clinical trial in MLL-rearranged leukemia showed target inhibition and evidence of bone marrow differentiation but modest antileukemic activity (18). A more recent therapeutic approach is disrupting the interaction of menin with MLL fusion proteins, which was shown to be a critical cofactor

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for MLL-mediated transformation. MI-503 and MI-463 (19) and VTP50469 (20) are inhibitors of the menin-MLL interaction, with preclinical efficacy against *in vivo* models of *MLL*-rearranged acute leukemia. Two phase I/II clinical trials of menin inhibitors KO-539 (NCT04067336) and SNDX-5613 (NCT04065399), an analogue of VTP50469, are ongoing in cases of AML with MLL rearrangement or the NPM1 mutation.

Chromatin immunoprecipitation-based screening in AML cells identified the gene encoding fibroblast growth factor 2 (*FGF2*) as a HOXA10 target gene. The MLL fusion protein Mll-Ell was shown to activate the FGF2 promoter in a HOXA9- and HOXA10-dependent manner resulting in the autocrine production of FGF2 (21). FGF2/FGFR inhibitors including kinase inhibitors, FGF ligand traps and small molecules that inhibit FGF2 binding to FGFRs are being evaluated for the treatment of different types of cancers, including leukemia (22, 23). This class of drugs may inhibit the expansion of myeloid progenitors mediated by HOXA 9 and 10 and a clinical trial (NCT03513484) is underway testing this concept.

RUNX1

The Runt-related transcription factor 1 (*RUNX1/AML1*) gene located on chromosome 21q22 is the most frequent target for chromosomal translocation in leukemia. In normal hematopoiesis, RUNX1 recruits its heterodimeric partner CBFβ to form the core binding factor (CBF) transcriptional complex that regulates differentiation, ribosome biogenesis, cell-cycle regulation, and TGF-beta signaling pathways. RUNX1 is indispensable for the establishment of definitive hematopoiesis, and heterozygous germline mutations result in a highly penetrant familial platelet disorder with a predisposition to AML (24).

The subset of favorable risk AML is typified by chromosomal rearrangements involving the CBF in AML. A translocation involving chromosomes 8 and 21 or inversion inv(16) (p13;q22) are found in 15% and 8% of AML cases, respectively, and both result in inhibition of wild-type RUNX1 through different mechanisms. In t(8;21), a corepressor, eight-twenty-one (ETO; ref. 25) is fused to the gene encoding the CBF subunit *RUNX1/AML1*. This fusion oncoprotein co-opts wild-type RUNX1/AML1 function and recruits epigenetic modifiers including histone deacetylases. Similarly, the chimeric protein CBFβ-SMMHC resulting from inversion 16 binds to and functions as a dominant-negative regulator of wild-type RUNX1 (26) and associates with HDAC8. In both cases, there is a dependency on histone deacetylation for transcriptional silencing leading to preclinical (27) and clinical studies (28) with the HDAC inhibitors in CBF AML.

In addition to translocations, somatic heterozygous *RUNX1* mutations have been detected in up to 20% of cases of myelodysplastic syndrome and AML with MDS-related changes and are associated with adverse outcome in AML (29). The main mutations in *RUNX1* described in AML are missense mutations found in the RUNT domain responsible for DNA binding and protein heterodimerization, and nonsense or frameshift mutations distributed throughout the entire gene that exert a dominant-negative effect on the transactivation activity. Approximately 30% of *RUNX1* mutations occur in combination or are associated with loss of heterozygosity, both of which lead to a complete loss of wild-type RUNX1. Increased RUNX1 inactivation in hematopoietic cells increases the propensity to develop leukemia, suggesting a dependency on RUNX1 protein dosage for disease onset (30).

A novel approach to target RUNX1 was the identification of compounds through a high-throughput screen to allosterically inhibit RUNX1 interaction with the oncogenic fusion protein CBFβ-SMMHC. One compound developed from this screen, AI-10-49, was shown to disrupt CBFβ-SMMHC binding to RUNX1 (31) and induced preferential apoptosis of inv (16) AML cells (32). This study provided important proof of concept for targeted therapies aimed at dysregulated transcription in specific genomic subsets of AML.

Although RUNX1 is generally considered to function as a tumor suppressor in the development of leukemia, wild-type RUNX1 is necessary to sustain leukemia caused by leukemogenic fusion proteins (33). The dependency on wild-type RUNX1 was therapeutically leveraged in a study (34) where suppression of RUNX1 was preferentially active against AML blasts expressing mutant RUNX1. It was shown that RUNX2 and RUNX3 (35) can compensate for RUNX1 silencing, simultaneous attenuation of all RUNX family members was more effective. The alkylating agent-conjugated pyrrole–imidazole polyamides, designed to bind to consensus RUNX-binding sequences, were active in a xenograft mouse model of AML (35), and this effect was mediated by p53 stabilization.

These studies on the pro-oncogenic role of RUNX1, while challenging the paradigm, are not well reconciled with clinical observation as disease course is not ameliorated in *RUNX1*-mutant AML with loss of heterozygosity at the wild-type allele. This dependency on the wild-type allele may be context dependent and lost in the setting of additional acquired mutations.

MYC

The ubiquitous proto-oncogene *MYC* transcriptionally regulates cell growth and metabolism. With progressive myeloid differentiation, there is a critical downregulation of MYC expression and ectopic expression blocks granulopoiesis. Epigenetic regulation at the *MYC* locus plays a critical role in myeloid differentiation and leukemogenesis. AML proteins are dependent on myc-induced self-renewal and survival (36, 37). The silencing of E2-2 by AML-ETO is an important mechanism for activation of *MYC* relevant to the clinical outcome of t(8;21) that can be reversed by overexpression of this transcription factor (38). In inv(16) AML, the impressive activity of AI-10-49 in the previous section was attributed primarily to the release of RUNX1 from CBFβ–SMMHC complex allowing replacement of chromatin remodeling complexes at three *MYC* distal enhancer elements (32). An additional ELN subset of AML with increased MYC expression is *CEBPα*-mutated (39) cases based on the negative regulation of MYC by CEBPα.

MYC has been directly implicated in myeloid leukemogenesis when ectopically overexpressed in murine bone marrow progenitor cells (40). High levels of MYC expression are associated with inferior survival outcomes (41) and contribute to chemoresistance in AML. One mechanism for this activity is the direct transcriptional regulation of the histone methyltransferase EZH2 (42).

Myc is challenging to target directly, given the absence of small-molecule binding pockets. One approach to surmount this hurdle has been targeting the transcription of this gene by destabilizing super-enhancer complexes. This has been accomplished using bromodomain-containing 4 (BRD4) inhibitors, which disrupt BETP binding to acetylated histones (43, 44), as well as proteolysis-targeting chimera (PROTAC) that direct BET proteins toward cereblon-mediated proteasomal degradation (45).

As a short half-life protein Myc also lends itself to strategies that inhibit translation. Homoharringtonine (HHT), an inhibitor of protein elongation, targets short-lived proteins such as Myc, Ctnnb1, and Mcl1 and has preferential activity in t(8;21) AML (46). Recent work shows the antileukemic effect of HHT is also linked to the drug binding to NKRF, a regulator of NF-κB activity, thereby affecting transcription of the *MYC* gene and lowering mRNA levels (47).

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Transcription Factor Activity Modulated by Cellular Localization

NF-kappa B

The transcription factor nuclear factor kappa B (NF-κB) comprises five members: Rel (c-Rel), RelA/p65, RelB, p50, and p52. Members of the NF-κB family exist in homo- or heterodimeric states and regulate the activation of specific target genes. In basal conditions, NF-κB proteins are predominantly cytoplasmic, associating with members of the inhibitory IκB family, which inhibit its DNA binding activity. Phosphorylation of Iκβ results in proteasomal degradation, allowing free NF-κB to enter the nucleus and activate transcription (48).

NF-kB is constitutively activated in AML $CD34^+$ cells and confers resistance to apoptosis (49). The importance of NF-kB as a target in leukemogenesis was shown by overexpression of mutant super-repressor IκB family members that attenuated AML development (50). Another study showed that activation of noncanonical NF-kB signaling by stabilization of NF-kB–inducing kinase (NIK) resulted in suppression of the canonical pathways and inhibited AML in animal models (51).

Several mechanisms have been identified for NF-kB upregulation in AML. CEBPα, as well as its mutant variants, interact with NFκB p50 and induce a subset of NFκB target genes, including prosurvival Bcl-2 and FLIP, through promoter binding (52). In t(8;21), loss of the c-terminal region of AML1 (RUNX1) prevents binding to, and inhibition of, IκB kinase (IKK), which results in NF-κB activation (53). *FL3-ITD*–mutated AML cells have enhanced activation of the threonine kinase TAK1 that serves as an important upstream modulator of NF-kappa (54). Also, FLT3 binds directly and activates IKK2, which induces the canonical NF-κB pathway (55). Another mechanism of upregulation in AML cells is Aurora kinase A, which activates IκB kinase complex through TRAF-interacting protein (TIFA), causing degradation of IκB. *In vivo* delivery of TIFA-inhibitory fragments potentiated the cytotoxicity of chemotherapy in murine xenografts (56).

High proteasome activity in AML patients positively regulates NF-κB activity (57) by degradation of ubiquitinated phosphorylated IκBα, and proteasome inhibitors exhibited selective toxicity for leukemic stem cells (58). These observations provided the rationale for the phase I/II clinical trials for the treatment of AML patients with proteasome inhibitor bortezomib in combination with chemotherapy (59) or hypomethylating agent decitabine (60) due to suppression of SP1/NF-κB–dependent DNA methyltransferase (61).

The anti-inflammatory agent choline–magnesium trisalicylate (62) was evaluated in combination with chemotherapy in a phase II randomized clinical trial. Gene-expression analysis of patient samples showed the use of this drug resulted in modulation of a subset of NF-κB target genes that were upregulated in response to chemotherapy (63).

Pevonedistat (MLN4924) inhibits NEDD8-mediated protein degradation by Cullin-RING ubiquitin ligases. This drug has shown promising clinical results in AML (64) and induces stabilization of IkBa, which inhibits nuclear NF-kappa in AML cells. Inhibition of the NFκB target superoxide dismutase 2 (SOD2) and resultant oxidative stress is critical to the activity of this drug (65).

Currently available TAK1 inhibitors 5z-7-oxozeaenol and AZ-TAK1 that block the phosphorylation and thereby the activity of TAK1 inhibited leukemic stem cells in an NF-kB–dependent manner (66). Another therapeutic approach utilizing the noncanonical NF-κB pathway is the use of SMAC mimetics which antagonize the inhibitors of apoptosis (IAP) family (67). By stimulating degradation of the c-IAP proteins, SMAC mimetics lead to accumulation of NF-κB–inducing kinase (NIK), and induction of NF-κB target genes such as TNFa, which in turn engages an autocrine/paracrine loop to trigger apoptosis.

Beta-catenin/TCF

In normal hematopoiesis, the Wnt/β-catenin pathway is active in primitive hematopoietic stem cells but downregulated with lineage commitment. In comparison, leukemic stem cells are dependent on canonical Wnt/β-catenin signaling for survival and self-renewal, suggesting that aberrant reactivation of β-catenin signaling is required for transformation. Inhibition of β-catenin phosphorylation status determines its nuclear localization, the unphosphorylated ("activated") form localizes to the nucleus where it binds to the members of the T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) transcription factor family, thereby converting them from transcriptional repressors into activators. Wang and colleagues (68) showed an increased level of unphosphorylated β-catenin in leukemic cells and deletion of β-catenin impaired *MLL-* or *HOXA9*-induced leukemia development in murine models, reinforcing its role as an oncogene in leukemia. The subset of post-MPN secondary AML also has increased beta-catenin stabilization (69), nuclear localization, and *TCF4* transcriptional cofactor activity due to upregulated AKT-mediated GSK3β phosphorylation and inactivation.

Beta-catenin is negatively regulated by the destruction complex comprising adenoma polyposis coli (APC), axin, glycogen synthase kinase β (Gsk3β), and casein kinase 1 (CK1). The subset of MDS/AML characterized by deletion 5q nearly always have loss of the *APC* gene resulting in hematopoietic stem cell exhaustion (70) mediated largely through a β-catenin–dependent mechanism (71). Conditional deletion of β-catenin rescued abnormal hematopoiesis in an *APC*-deficient murine model. Another important negative regulator of β-catenin located in the common deleted region for del(5q) is casein kinase 1A1 (*CSNK1A1*) (72). These observations fueled attempts to target β-catenin using indomethacin or β-catenin–specific shRNAs resulting in selectively inhibition of del(5q) leukemia cells (73). Inhibitors of CK1 also offer therapeutic promise in this AML subset.

There is also a role for cell-extrinsic Wnt signaling in the hematopoietic niche cells (74). Conditional activation of β-catenin signaling within the osteoblast lineage in mice induces overexpression of the Notch ligand *Jagged 1*. The consequent Notch signaling upregulation causes clonal expansion of hematopoietic cells leading to AML.

The interaction between β-catenin and TCF transcription factor is a critical node for Wnt signaling. Peptide inhibitors of this interaction reduced β-catenin–TCF transcriptional activity and decreased the viability of Wnt-dependent cancer cells (75). More recent work shows preclinical therapeutic activity of peptoids, peptidomimetic oligomers that mimic protein secondary structure motifs, to disrupt the β-catenin–TCF interaction (76). A recent approach is the use of small-molecule inhibitor BC2059 to disrupt the binding of the scaffolding protein TBL1 to beta-catenin. TBL1 binds to cytoplasmic β-catenin, protecting it from ubiquitination, and nuclear beta-catenin to facilitate the transcriptional activity of TCF4. Treatment with BC2059, by inducing depletion of nuclear and cytoplasmic β-catenin levels disrupted the transcriptional activity of β-catenin–TCF4. This inhibited growth and survival of leukemia-initiating AML stem-progenitor cells in secondary AML (77). In addition, a proteomic analysis of the β-catenin binding partners in the nuclear and cytoplasmic compartments of leukemia cells (78) revealed the transcriptional partner LEF-1 played an important role in nuclear retention of

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β-catenin. Small-molecule inhibitors of the β-catenin:TCF/LEF interaction are under development.

FOXM1

FOXM1 is an oncogenic transcription factor of the Forkhead family with an important role in proliferation and cell-cycle progression. FOXM1 is preferentially expressed in proliferating and malignant cells compared with quiescent or differentiated cells. It is negatively regulated by major tumor suppressors, including RB, p53, and p19. FOXM1 has been implicated in diverse cellular processes encompassing all the hallmarks of cancer, including tumorigenesis, cell proliferation, metastasis.

Recent work (79) showed that FOXM1 is essential for maintaining the quiescence and self-renewal capacity of MLL-AF9 transformed leukemic stem cells *in vivo* by binding to and increasing nuclear beta-catenin in this AML model. FOXM1 was found to be overexpressed in FLT3-ITD AML cases (80) and its expression was induced in response to FLT3 ligand treatment of leukemia cells. These are both high-risk AML subsets that could benefit from inhibiting the activity of this transcription factor.

FOXM1 is dependent on its nuclear localization to function as a transcription factor, and its protein interactions have been shown to regulate this localization. It has previously been shown that across multiple tumor types, NPM binds to FOXM1 (81) and their interaction is required for sustaining the level and nuclear localization of FOXM1. The relevance of FOXM1 to AML therapeutic outcomes was recently demonstrated by linking the chemosensitivity conferred by the *NPM1* mutation in AML to the cytoplasmic relocalization and consequent inactivation of the oncogenic transcription factor FOXM1 (81, 82). Nuclear FOXM1 was then validated as an independent clinical predictor of chemotherapy resistance in AML (83), and its overexpression in transgenic murine AML models was shown to directly impart chemoresistance. Potentiating chemotherapy by inhibiting FOXM1 could theoretically be applicable all cases with wild-type *NPM1* where FOXM1 would be nuclear.

Peptide inhibitors of FOXM1 have been shown to have antileukemic activity in preclinical models (79, 84). Proteasome inhibitors have been shown to target FOXM1 (85) by stabilizing HSP70, which is a negative regulator of FOXM1 (86). In binding to FOXM1, HSP70 inhibits the DNA binding of FOXM1 and its transcriptional activity. Clinically approved proteasome inhibitors ixazomib and bortezomib inhibit FOXM1 and sensitize AML cells to chemotherapy in preclinical studies (82).

It has previously been shown that the mitotic kinase MELK binds and phosphorylates the oncogenic transcription factor FOXM1 in glioblastoma stem-like cells (GSC; ref. 87). A novel MELK inhibitor OTS167 is being evaluated in the treatment of relapsed leukemia and has been shown to suppress FOXM1 (88). High-throughput screening lead to the identification of FDI-6 (89), a novel small-molecule inhibitor of FOXM1 that blocks DNA binding. It has been shown to bind directly to FOXM1, displacing it from its genomic targets in breast cancer.

PU.1

The Ets-domain transcription factor PU.1 plays an important role in hematopoietic stem cell maintenance by regulating differentiation, proliferation, and survival (90). It was recognized over a decade ago that PU.1 cooperated with interferon regulatory factors (Irf2 and Irf8) to activate a set of phagocyte-specific genes in neutrophils and monocytes (91). More recently, it was shown that PU.1 cooperates with CEBPA and RUNX1 to direct myeloid differentiation. Although loss-of-function mutations/translocations of *CEBPA* and *RUNX1* are well recognized in AML, recurrent alterations to PU.1 have not been found. However, interaction with NPM is an important determinant of PU.1 activity, and *NPM1* mutations in AML have been shown to delocalize PU.1 to the cytoplasm (92). By dislocating PU.1 into the cytoplasm, mutant NPM disrupts the PU.1/CEBPA/RUNX1 transcription factor complex, thereby uncoupling proliferation from differentiation. Importantly, this differentiation arrest was reversed by clinical small molecules that inhibit nuclear export such as selinexor or depletion of corepressor DNMT1 from the CEBPA/RUNX1 complex, a finding that opens the door to differentiation therapies for patients with *NPM1*-mutant AML.

Another study indicates the novel function of PU.1 in cell death responses to clinically relevant drugs in AML cells (93). PU.1 significantly prevents NF-κB–mediated upregulation of antiapoptotic genes such as *cFLIP*, *MCL-1*, *BCL-2*, and *BCL-XL* upon TRAIL treatment. Moreover, PU.1 deficiency significantly increased AML cell resistance to anthracycline treatment. These results reveal a new facet of PU.1's tumor suppressor function during antileukemic therapies.

Overexpression of PU.1 is sufficient to trigger differentiation and apoptosis of AML samples (94) but difficult to achieve pharmacologically. Paradoxically, Debre and colleagues suggest that elimination of PU.1 has therapeutic benefit in leukemia (95) based on the observation that complete loss of PU.1 leads to stem cell failure (96). Small molecules of the heterocyclic diamidine family were developed to allosterically interfere with PU.1-chromatin binding through interaction with the DNA minor groove that flanks PU.1-binding motifs. These small molecules disrupted the interaction of PU.1 with target gene promoters causing downregulation of canonical PU.1 transcriptional targets and decreased tumor burden resulting in increased survival of AML xenotransplant models. Additional experiments should reconcile these opposing effects of manipulating PU.1 in AML.

Transcription Factor Mutations Causing Loss of Function

p53

The tumor suppressor p53 has a critical role in DNA damage response by inducing cell-cycle arrest to enable repair or consigning unsalvageable cells to apoptosis. *TP53* mutations are present in <10% of *de novo* AML patients (97) but is enriched in therapy-related AML (t-AML) with a prevalence of 30% to 40% (98, 99). Moreover, in four cases of t-AML, the *TP53* mutation predated the development of disease or chemotherapy exposure (100). These data suggest a model in which rare hematopoietic stem and progenitor cells carrying age-related *TP53* mutations are resistant to chemotherapy and expand preferentially after treatment. This observation was reiterated in a murine Fanconi anemia model ($FNCC^{-/-}$) where haploinsufficiency of *TP53* in the Fanconi anemia mice ($FNCC^{-/-}/TP53^{+/-}$ mice) resulted in the rapid onset of AML in response to inflammatory stress and evidence of accumulation of DNA breaks and radials in the bone marrow (101).

TP53 mutations in *AML* have a 2-fold effect of loss of transactivating function of p53 and downregulation of the ubiquitin ligase MDM2 which results in stabilization of mutant protein (102). This results in increased microsatellite instability in multiple loci in 94% of AML patients with *TP53* mutations (99). Recent studies utilized CRISPR-Cas9 to establish a dominant-negative effect influences clonal selection of *TP53* mutations in myeloid leukemias (103).

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In AML cells while the incidence of *TP53* mutations is low, dysregulation of p53 function appears to be a frequent event (104) mediated through overexpression of canonical negative regulators such as MDM2 and/or its homolog MDM4 or p14ARF inactivation. Proteomic studies confirm MDM2 overexpression occurs in over a third of patients with *TP53* wild-type AML resulting in very low levels of p53 protein and an outcome similar to *TP53*-mutant cases.

In an alternate paradigm, a recent study identified a neomorphic function of the *R175H* mutation, a mutant form of *TP53* that represents the most common allele in patients with AML. This mutant p53 exerts its effect by enhancing cellular self-renewal in HSPCs through a novel mediator, FOXH1. Suppression of either mutant p53 or FOXH1 ablated this stemness capacity by triggering differentiation (105).

AMG 232 is an oral, selective MDM2 inhibitor that restores p53 tumor suppression by blocking the MDM2–p53 interaction (106). This drug was well tolerated as monotherapy and in combination with a MEK inhibitor in relapsed/refractory AML (107). It was shown to upregulate p53 transcriptional targets in correlative studies and responses were restricted to *TP53* wild-type AML.

APR-246 is a first-in-class small molecule that covalently binds to the p53 core domain, modifies cysteine residues inducing thermodynamic stabilization, which can restore wild-type activity. The first-in-human study with this compound showed clinical activity in an AML patient with a somatic *TP53* core domain mutation. Preclinical studies in patient AML samples showed APR-246 reactivates the p53 pathway and can induce an apoptosis program and downregulate oncogenic FLT3 signaling (108). Clinical phase II studies of APR-246–based combination therapy demonstrated unprecedented response rates of over 80% in *TP53*-mutant MDS and AML (109, 110). The randomized phase III study of APR-246 and azacitidine versus azacitidine in *TP53*-mutant MDS patients has rapidly completed accrual (NCT03745716) and the drug has been granted breakthrough therapy designation for this indication by the FDA.

CEBP-α

The CCAAT enhancer-binding protein alpha (*CEBP-α*) is a tumor suppressor gene located within 19q13.1 that encodes a transcription factor involved in granulocytic differentiation. The *CEBP-α* mutation is considered a primary event in leukemogenesis and shows three patterns: single allele mutated, biallelic mutations involving an N-terminal and a C-terminal, and homozygous mutation due to loss of heterozygosity. Mutations in the N-terminus of *CEBP-α* cause frameshift and dominant p30 translation which has less transcriptional activity and acts in a dominant-negative fashion. The mutations in the C-terminus of *CEBP-α* reduce its DNA binding activity (111). CEBPα is mutated in 10% to 15% of AML cases and can be functionally suppressed in other AML subsets, such as transcriptional repression by the leukemic fusion protein AML1-ETO, post-translational modifications by the oncogenic kinase FLT3-ITD, and protein degradation by the tribbles homologs, Trib1 and Trib2.

Myeloid leukemia factor 1(MLF1) was recently identified (112) as a naturally occurring inhibitory protein for the E3 ubiquitin ligase complex, Trib1-COP1, which targets CEBP-α, for degradation. Transcriptional enhancement of *MLF1* expression and/or increased nuclear compartmentalization of MLF1 represent potential strategies for leukemia therapy.

Identification of small molecules that can restore CEBP-α activation is a therapeutic strategy in AML. High-throughput screening led to the identification of ICCB280, a quinazolinone derivative, as an inducer of CEBP-α (113). Treatment with this compound induced morphologic and functional evidence of myeloid differentiation in AML cells. ICCB280-induced CEBP-α and its downstream targets, such as CEBP-ε and G-CSFR, at both the mRNA and protein levels and downregulated MYC, which is negatively regulated by CEBP-α. CEBPα–p30 requires interaction with cofactor Wdr5 to inhibit myeloid differentiation. The small-molecule OICR-9429 was able to inhibit this interaction, resulting in selective inhibition and differentiation of p30-expressing human AML cells (114).

Conclusion

Transcription factors contribute to stem cell maintenance, determination of differentiation, and maturation of HSPCs. Mutations, translocations, or aberrant expression of transcription factors can

Table 1. Targeted therapies in AML.

WHO classification	Oncogene activation	Loss of tumor suppressor	Targeted therapies	Mechanism of action
Inv(16)	MYC	RUNX1	AI-10-49	Disrupt CBFβ–SMMHC binding to RUNX1
t(8;21)	MYC	RUNX1	Homoharringtonine	Interferes with myc protein elongation
	NF-KB		Panabinostat, romidepsin	Degradation of AML–ETO fusion protein and myeloid differentiation
MLL (11q23) and HOXA rearrangement	HOXA		EPZ-5676/pinometostat	DOT1L inhibitor
			VTP50469, MI503, MI463	Inhibits interaction of menin with MLL
			Nintedanib	Inhibits HOXA-mediated FGFR signaling
5q-	β-catenin		BC2059	Disrupts binding of TBL1 to beta-catenin
NPM1 ^{mut}	HOXA	PU-1	Selinexor	Nuclear export inhibitor
			DB2115 or DB2313	Inhibit chromatin binding of PU.1
CEBPα$^{-/-}$	MYC	CEBP-α	ICC280	Inducer of wild-type CEBPα
			OICR-9429	Inhibits mutant CEBPα binding to cofactor Wdr5
P53 ^{mut}		p53	APR-246	Binds p53 core domain restoring WT function
FLT3ITD	NF-KB		Proteasome inhibitor pevonedistat	Induces stabilization of IkBα, which inhibits NF-K
RUNX1 ^{mut}	RUNX1		Drug-conjugated pyrrole–imidazole polyamides	Inhibit RUNX binding to DNA and are conjugated to alkylator chlorambucil

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cause malignant transformation of hematopoietic cells (**Fig. 1**). In addition, transcriptional plasticity (115) allows malignant cells to use alternative enhancers to sustain the expression of key survival genes.

Transcription factors have not been traditionally considered "druggable," as they lack enzymatic activities and the DNA binding pockets and protein–protein interaction domains are generally shallow. There have been advances in direct inhibition of transcription factors with the development of PU.1 and RUNX inhibitors and small molecules that blocks the interaction between beta-catenin and its activator TBL1 (**Table 1**). Insights into critical dependencies of transcription factors, whether it is activation of downstream kinases such as FGF in *MLL*-rearranged AML or stabilizing protein interactions in the case of menin-MLL, uncover new therapeutic possibilities.

By linking the WHO genomic classification of AML to subtype-specific salient transcription factor deregulations, we highlight novel strategies to integrate into the treatment approach of AML patients (**Table 1**).

Authors' Disclosures

No disclosures were reported.

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